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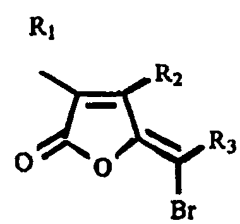
(54) Title: INHIBITION OF GRAM POSITIVE BACTERIA

(57) Abstract

A method of inhibiting the growth of a Gram positive bacterium, the method comprising treating the bacterium with an effective amount of one or more furanones having the Formula as set out in the Figure, wherein the effective amount of the one or more furanones does not substantially adversely effect the survival of an animal cell when exposed to the one or more furanones.



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Inhibition of Gram positive bacteria

Technical Field

The present invention relates to the use of furanone compounds as antibacterial agents, particularly as antibacterial agents for Gram positive bacteria.

Background Art

It is known that a variety of furanone compounds possessing antifungal and antimicrobial properties can be isolated from red marine algae *Delisea fimbriata*. *Delisea elegans* and *Delisea pulchra* (Reichelt and Borowitzka (1984) Hydrobiologia 116: 158-168). When first isolated, it was thought that these compounds may be suitable as antimicrobial agents for use in animals including humans. Unfortunately, it was found that most if not all of these naturally occurring compounds were toxic to animal cells at the concentrations required to inhibit microorganisms and therefore unsuitable for many veterinary and medical applications.

Gram positive bacteria are a major problem in hospitals, on skin, in the dental area. for heart transplants, catheters, and other biomedical implants. Unfortunately, not all antimicrobial agents are active against Gram positive bacteria. Gram positive bacteria are also present in domestic areas like bathrooms. toilets and kitchens and can also cause a disease hazard for these sources. Accordingly, there is a need for more agents that are suitable to inhibit or kill these types of microorganisms in many varied situations including domestic, veterinary and medical applications.

The present inventors have now made the surprising finding that new synthetically produced furanone compounds have inhibitory activity against Gram positive bacteria without having corresponding deleterious activity against animal cells previously reported to be the case with naturally occurring furanones.

Disclosure of Invention

In a first aspect, the present invention consists in a method of inhibiting the growth of a Gram positive bacterium, the method comprising treating the bacterium with an effective amount of one or more furanones having the Formula as set out in Figure 1, wherein the effective amount of the one or more furanones does not substantially adversely effect the survival of an animal cell when exposed to the one or more furanones.

In a preferred embodiment of the first aspect of the present invention, the furanone has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45, mixtures and/or racemic mixtures thereof as shown in Figure 2.

Preferably, the furanone is selected from is compound 2, 30, 45 or a mixture of compounds 33/34. More preferably, the furanone is a mixture of compounds 33/34 or compound 2.

Although compounds 33/34 have been found to be particularly suitable as antimicrobial agents, it will be appreciated, however, that other furanones may have similar or even greater activity against Gram positive bacteria. The finding by the present inventors that furanones other than the known naturally occurring compounds (for example compound 4) have activity against Gram positive bacteria without being substantially toxic to animal cells may lead to the production and use of other furanone compounds. Accordingly, it will be appreciated that the present invention covers other such compounds.

The present inventors have found that a concentration of a furanone of about 500 ng/ml was effective against a Gram positive bacterium. Higher concentrations were also effective but importantly were not toxic to animal cells. It will be appreciated that even lower concentrations may also be active against certain Gram positive bacteria and the present invention is not limited to the concentrations as tested and described herein.

The concentrations of furanones found to be active against Gram positive bacteria are not inhibitory against the Gram negative bacteria presently tested by the present inventors. The activity of the furanones are therefore surprisingly selective in their inhibitory action for Gram positive bacteria.

In a second aspect, the present invention consists in the use as an inhibitory agent against Gram positive bacteria of an effective amount of a furanone having the Formula as set out in Figure 1, wherein the effective amount of the furanone do not substantially adversely effect the survival of an animal cell when exposed to the furanone.

In a preferred embodiment of the second aspect of the present invention, the furanone has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

Preferably, the furanone is selected from is compound 2, 30, 45 or a mixture of compounds 33/34. More preferably, the furanone is a mixture of compounds 33/34 or compound 2.

The active furanones can be used as antibacterial agents for direct administration to mammals; as additives or preservatives for medical/surgical devices, disinfectants, soaps, shampoos, hand washes, denitrifies, household cleaning formulations, detergents for laundry and dishes; in wash and treatment solutions for topical use, instruments and devices including contact lenses, and other disinfecting and antibacterial applications

The active furanones can be formulated as an antiseptic, disinfectant or antimicrobial agent. It will be appreciated that the formulations will be particularly useful in situations where it is necessary to inhibit or kill Gram positive bacteria without causing any deleterious effects on animal or mammalian cells.

In a third aspect, the present invention consist in a method of inhibiting the growth of a Gram positive bacterium in a subject infected with the bacterium, the method comprising administering to the subject an effective amount of one or more furanones having the Formula as set out in Figure 1, wherein the effective amount of the one or more furanones do not substantially adversely effect cells of the subject.

In a preferred embodiment of the third aspect of the present invention, the furanone has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

Preferably, the furanone is selected from is compound 2, 30, 45 or a mixture of compounds 33/34. More preferably, the furanone is a mixture of compounds 33/34 or compound 2.

In a fourth aspect, the present invention consists in a pharmaceutical composition active against Gram positive bacteria, the composition including one or more furanones having the Formula as set out in Figure 1 together with one or more pharmaceutically acceptable diluents or excipients, wherein the one or more furanones do not substantially adversely effect animal cells at the inhibitory concentration used in the composition.

In a preferred embodiment of the fourth aspect of the present invention, the furanone has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

Preferably, the furanone is selected from is compound 2, 30, 45 or a mixture of compounds 33/34. More preferably, the furanone is a mixture of compounds 33/34 or compound 2.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described in the following examples with reference to the accompanying drawings.

Brief Description of Drawings

Figure 1 shows the general structure of furanones suitable for the present invention wherein R_1 is a hydrogen atom, a hydroxyl, ester or an ether group and wherein R_2 and R_3 are each a hydrogen atom or a halogen atom.

Figure 2 shows the structure of furanone compounds formula 2, 24, 25, 26, 27, 30, 33, 34, 45 tested for activity against Gram positive bacteria.

Figure 3 shows the results of the effect of furanone compounds 2, 24/25 and 30 on the growth of *Staphylococcus aureus* 29.

Figure 4 is shows the results of the effect of furanone compounds 2, 24/25 and 30 on the growth of *S. aureus* 015.

Figure 5 is shows the results of the effect of furanone compounds 2, 24/25 and 30 on the growth of *S. epidermidis* 017.

Modes for Carrying Out the Invention

METHODS AND RESULTS

Furanone compounds

A number of furanone compounds having structures shown in Figure 2 were tested directly on Gram positive bacteria. Synthetically produced furanones are often produced as mixtures of isomers (e.g., 33/34, 26/27, etc.). It will be appreciated, however, that variations in these structures are similar enough to be expected to have an effect, and thus the present invention includes within its scope furanones with the more general structure shown in Figure 1 which do not substantially adversely effect the survival of an animal cell when exposed to the furanone.

Microbiology

An initial screening of six different furanones against growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* was performed in a BioRad 3550 Microplate Reader. Ten percent (10%) of an overnight culture (10^8 cells/ml, resulting in final cell densities of 10^6 cells/ml) was added to the growth media. Nutrient Broth, containing furanones at the concentrations 10 and 1 μg/ml. The bacteria were incubated at 37°C and growth was measured at 595 nm for 9 hours. Cytotoxicity in mammalian systems was measured as inhibition of the growth of mouse fibroblast cells.

The results showed several important features. First, the furanone compounds strongly inhibited growth at effective concentrations (e.g., 1 and 10 ug/ml) comparable to that of standard commercial antibiotics. Second, synthetic furanone compounds or mixtures of furanone compounds (e.g., 30; 24/25; 26/27; 33/34) were often more active than those furanone compounds which are produced naturally by the red algae *Delisea pulchra*, the main natural source of these fimbrolides. Third, a number of these furanone compounds were inhibitory at concentrations well below concentrations that inhibited mouse fibroblasts. That is, the furanone compounds inhibited bacteria at non-toxic (to mammals) concentrations. For example, compounds 30 and 33/34 were the most active against both *Staphylococcus aureus* and *Staphylococcus epidermidis* (Table 1), completely inhibiting growth at 10 ug/ml. However, cultured mouse cells were not affected by compound 33/34 (for example) until 50 ug/ml.

Table 1. Activity of six different furanones against growth (after 9 hr) of two Gram positive bacteria and a mouse fibroblast cell line.

	Staphylococcus aureus		*Staphylococcus epidermidis*		Mouse cell line
Compound	10 µg/ml	1 µg/ml	10 µg/ml	1 µg/mg	(µg/ml)[a]
*33/34	0	73.0[b]	0	80.0	50
*30	0	56.6	62.2	72.1	20
*26/27	77.4	82.1	19.9	71.6	5
*24/25	58.2	80.1	47.7	62.2	50
#4	34.3	84.2	53.2	77.1	1
#2	57.9	87.9	53.2	63.7	150

a Lowest concentration of furanones which significantly inhibited the growth of mouse fibroblast cell line L292. A 30% depression of growth is deemed significant

b % growth compared to the control value

* synthetically produced furanones

\# natural products

The tests described above, while clearly demonstrating the efficacy of these compounds - particularly the synthetic furanones - against Gram positive bacteria, are unrealistic for many veterinary and medical applications in that they combine a very nutrient rich media with a very high initial inoculum density. In many clinical situations, colonising bacteria are much less dense initially, or will invade or contaminate much less nutrient rich media (e.g., saline). Thus a series of tests were done with a lower density innocula, or in less nutrient rich media. These experiments used compound 33/34, the most active synthetic mix in the initial screen.

Low numbers of overnight cells (*Staphylococcus aureus*) were inoculated to three different media; Nutrient broth (NB), and 10% and 5% of NB each diluted in 0.9% NaCl solution containing compound 33/34. This gave a final amount of 10 cells/ml media. The samples were incubated at 37°C and growth was measured at 610 nm. Three different concentrations of compound 33/34 were tested, 500 ng/ml, 1 µg/ml and 5 µg/ml. Results reported are for 8 days (192 hr) and 19 days (456 hr) are shown in Table 2.

Table 2. Growth of *Staphylococcus aureus* (final amount of 10 cells/ml) in various media containing compound 33/34.

Medium	Control	Concentration of compounds 33/34 500 ng/ml	1 μg/ml
Nutrient broth (NB)	8 h[a]	growth after 30 hr	c
10% NB[d]	45 h	b	c
5% NB[d]	61 h	b	c

a Time taken for control cultures to initiate growth
b No growth after 192 hr
c no growth after 456 hr
d NB media diluted with 0.9% NaCl solution

Table 2 shows clearly that in these realistic growth conditions, concentrations of 33/34, as low as 500 ng/ml, completely inhibited the growth of *S. aureus*. These concentrations are two orders of magnitude less than those which affect mouse cell lines. The inhibitory concentrations are also lower than those used for any current commercial antibiotic against Gram positive bacteria.

Occular Applications

It has been reported that natural furanones have ability to inhibit the adhesion and swarming in a range of marine bacteria at concentrations that did not effect growth. In order to investigate whether different side chain would play any anti-bacteria roles, the effect of synthetic fuanones (at lower concentrations) on growth of different ocular bacterial strains were examined.

MATERIALS AND METHODS

Bacteral strains

Bacterial strains included the commonly isolated ocular microorganisms listed in Table 3.

Table 3. Ocular gram-positive bacteria used in the study

Strain	Gram stain	Source
S. aureus 001	Gram-positive cocci	Type strain
S. aureus 015	Gram-positive cocci	Type strain
S. aureus 029	Gram-positive cocci	CLPU
S. epidermidis 017	Gram-positive cocci	CLARE
Corynebacterium 003	Gram-positive rods	Infiltrative keratitis

Furanone compounds

Compounds 2, 24/25, 26, 30, 33/34 and 45 were examined for their effects on the growth of the bacterial strains. The concentrations used in the study for all the tested compounds were within the non-cytotoxic ranges.

Effect of furanone compounds on the growth of bacteria in TSB

Strains were cultured in 5 ml of TSB with or without SUs in 35° C water bath with agitation (120 rpm). Growth rates were monitored by reading OD_{660nm} at time intervals of 1, 2, 3, 4, 5, 7, and 24 hours.

Effect of furanone compounds on the growth of bacteria in artificial tear fluid (AFT)

The formulation of ATF used in the study was reflected in the composition of closed-eye tears. It contained 2% (w/v) of human serum.

Overnight growing bacteria were harvested and washed once with PBS. After resuspending in ATF to OD_{660} 0.1 (10^8 cells/ml), 200 μl of bacterial cell suspension was mixed with a same volume of ATF containing furanone compounds in a Bijio bottle. The final concentration of furanone compounds was 20 μg/ml. The cultures were then incubated at 35° C for 24h. Viable counts were quantified by plating out serial dilution of the ATF culture onto nutrient agar.

Minimum inhibition concentration of furanone compounds

One hundred μl/well of TSB containing two-fold serial diluted furanone compounds (at the concentration range from 20 to 1.25 μg/ml) were distributed in 96 well of microtitre plate. Ten μl of 10^4cells/ml overnight bacterial culture was added in each well to get the final concentration of cell numbers of 10^3 cells/ml. After incubation for one or two days at 35°C, bacterial growth was measured at Abs_{450nm} in a microtitre reader.

RESULTS

Effect of furanone compounds on the growth of Gram positive bacteria in TSB

The growth curves showed in Figures 3 to 5 demonstrated that furanone compound 2 and furanone compounds 24/25 inhibited the growth of *S. aureus* and *S. epidermidis* strains at the tested concentrations of 100 μg/ml and 20 μg/ml respectively; furanone compound 30 only slowed down the growth of *S. aureus* and *S. epidermidis* strains at lower concentrations (10 μg/ml. Figure 5). There were only less than 8% growth of *S. aureus* strains after 24 h incubation time in the presence of furanone compounds 2 and 24/25 (Table 4). For Gram positive rods *Corynebacterium sp 003*, furanone compound 2 inhibited approximately 86% of growth at 100 μg/ml. Furanone compounds 24/25 (20 μg/ml) and 30 (10 μg/ml) only slowed down the growth of *Corynebacterium sp* 003 (Table 4), as the growth reached 71 and 90% in the presence of furanone compounds 24/25 (20 μg/ml) and 30 (20 μg/ml) respectively. after 24 h incubation.

Table 4. Percentage growth of Gram positive bacteria in the presence of furanone compounds compared to the control values (after 24h).

Strain	furanone compound 2 100 μg/ml	furanone compounds 24/25 20 μg/ml	furanone compound 30 10 μg/ml
G^+ cocci:			
S. aureus 015	8	5	97
S. epidermidis 017	7	3	91
G^+ rods:			
Corynebacterium 003	14	71	90

Low numbers of overnight cells (*Staphylococcus aureus*) were inoculated to 5% NB diluted in PBS (buffered NaCl solution) containing furanone compounds 33/34. This gave a final amount of 25 cells/ml media. The samples were incubated static at room temperature and growth was measured by CFU (colony forming unit). One concentration of furanone

compounds 33/34 was tested, 5 μg/ml. Results reported are for 2 days, 5 days and 38 days and reported in Table 5.

Table 5. Growth of *Staphylococcus aureus* (final number of cells/ml) in 5% NB media containing furanone compounds 33/34.

Time (days)	Control	Furanone compounds 33/34 (5 ug/ml)
2	10^5 cells/ml	no growth
5	10^4 cells/ml	no growth
38	10^3 cells/ml	no growth

The results in Table 5 shows that growth of *S. aureus* was clearly inhibited at concentration 5 μg/ml.

A screening of six different furanone compounds against growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus salivarius*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Micrococcus luteus* was performed in a BioRad 3550 Microplate Reader. Ten percent of overnight cultures were added to the growth media, NB containing furanones at the concentration 10 μg/ml. The bacteria were incubated at 37°C and growth was measured at 595 nm for 24 hours. The results are shown in Table 6.

Table 6. Activity of different furanone compounds (at concentration of 10 ug/ml) against growth (after 24 hr) of six Gram positive bacteria. Results given as % growth compared to the control value.

Furanone Compound	*Staph. aureus*	*Staph. epidermidis*	*Strep. salivarius*	*Strep. pyogenes*	*Entero. faecalis*	*Micro. luteus*
45	4	22	69	26	85	100
33/34	0	0	0	0	62	63
30	0	62	73	50	54	86
26/27	77	20	92	84	75	85
24/25	58	48	87	72	92	65
4	34	53	85	0	75	75
3	22	32	86	14	89	75
2	58	53	86	87	77	86

Table 6 shows that all the different furanone compounds tested inhibit the growth of Gram positive bacteria and that the furanones act in a species specific manner. However, compound 33/34 demonstrated a broader range of inhibitory activity compared to the other compounds and also completely preveneds growth of four Gram positive bacteria.

DISCUSSION

Prior art (Reichelt and Borowitzka (1984) Hydrobiologia 116: 158-168) showed an initial indication that naturally produced furanones can inhibit the growth of Gram positive bacteria at probably relatively high concentrations. However, the authors concluded that furanones were not useful as antibacterial agents in mammalian systems because they were toxic. The investigators based these conclusions primarily on tests with crude extracts of *Delisea pulchra* in which compound 4 is one of the more abundant furanones. The present inventors found compound 4 to be quite toxic against mammalian cell lines but determined that a number of other furanone compounds are necessarily toxic to mammalian cells.

Surprisingly, the present inventors have shown that the results with compound 4 are not generally representative of the effects of other furanone compounds. which are for the most part strongly inhibitory against Gram positive bacteria at non-cell line inhibitory concentrations. Moreover, synthetic furanone compounds tested were more effective than naturally occurring compounds.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A method of inhibiting the growth of a Gram positive bacterium, the method comprising treating the bacterium with an effective amount of one or more furanones having the Formula as set out in Figure 1, wherein the effective amount of the one or more furanones does not substantially adversely effect the survival of an animal cell when exposed to the one or more furanones.

2. The method according to claim 1 wherein the one or more furanones has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

3. The method according to claim 2 wherein the one or more furanones is the mixture of compounds 33/34.

4. The method according to claim 2 wherein the one or more furanones is compound 2.

5. The method according to any one of claims 1 to 4 wherein the concentration of the one or more furanones is about 500 ng/ml.

6. Use as an inhibitory agent against Gram positive bacteria of an effective amount of one or more furanones having the Formula as set out in Figure 1. wherein the effective amount of the furanone do not substantially adversely effect the survival of an animal cell when exposed to the furanone.

7. The use according to claim 6 wherein the one or more furanones has the formula 2. 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

8. The use according to claim 7 wherein the one or more furanones is a mixture of compounds 33/34.

9. The use according to claim 7 wherein the one or more furanones is compound 2.

10. The use according to any one of claims 6 to 9 of one or more furanones as antibacterial agents for direct administration to mammals; as additives or preservatives for medical/surgical devices, disinfectants, soaps, shampoos, hand washes. denitrifies, household cleaning formulations, detergents for laundry and dishes; in wash and treatment solutions for topical use, instruments and devices including contact lenses, and other disinfecting and antibacterial applications.

11. The use according to any one of claims 6 to 9 of one or more furanones formulated as an antiseptic, disinfectant or antimicrobial agent.

12. A method of inhibiting the growth of a Gram positive bacterium in a subject infected with the bacterium, the method comprising administering to the subject an effective amount of one or more furanones having the Formula as set out in Figure 1, wherein the effective amount of the one or more furanones do not substantially adversely effect cells of the subject.

13. The method according to claim 12 wherein the one or more furanones has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

14. The method according to claim 13 wherein the one or more furanones is the mixture of compounds 33/34.

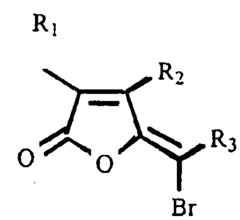
15. The method according to claim 13 wherein the one or more furanones is compound 2.

16. A pharmaceutical composition active against Gram positive bacteria, the composition including one or more furanones having the Formula as set out in Figure 1 together with one or more pharmaceutically acceptable diluents or excipients, wherein the one or more furanones do not substantially adversely effect animal cells at the inhibitory concentration used in the composition.

17. The composition according to claim 16 wherein the one or more furanones has the formula 2, 24, 25, 26, 27, 30, 33, 34, 45 mixtures and/or racemic mixtures thereof as shown in Figure 2.

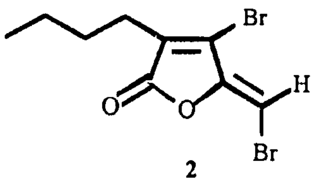
18. The composition according to claim 17 wherein the one or more furanones is the mixture of compounds 33/34.

19. The composition according to claim 17 wherein the one or more furanones is compound 2.



wherein R_1 is a hydrogen atom, a hydroxyl, ester or an ether group and wherein R_2 and R_3 are each a hydrogen atom or a halogen atom.

Figure 1



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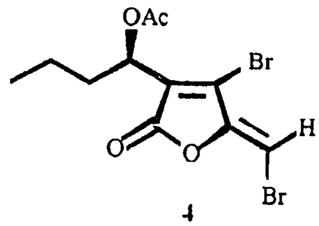

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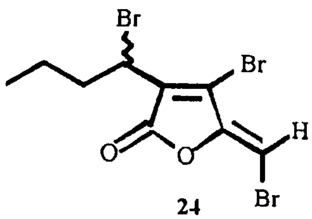

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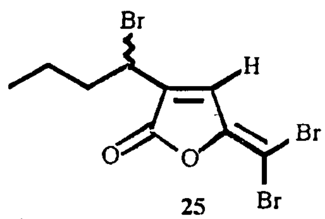

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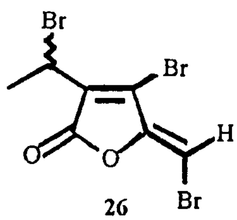

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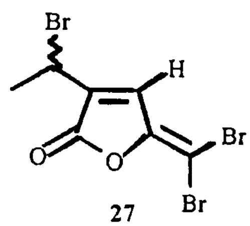

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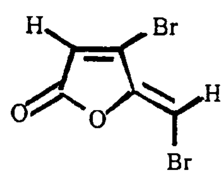

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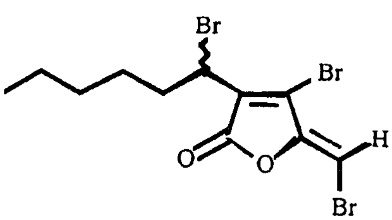

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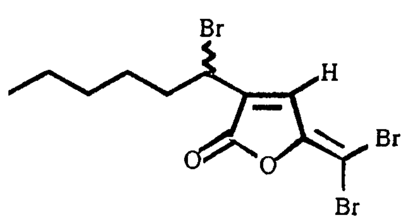

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Figure 2

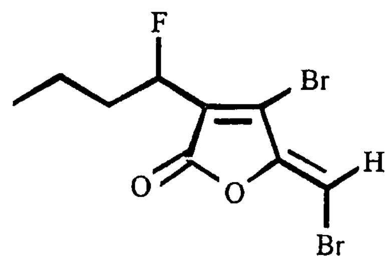

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Figure 2 (cont'd)

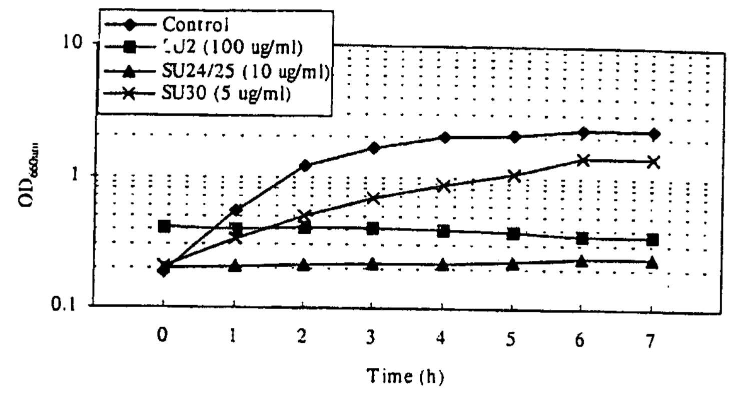

Figure 3

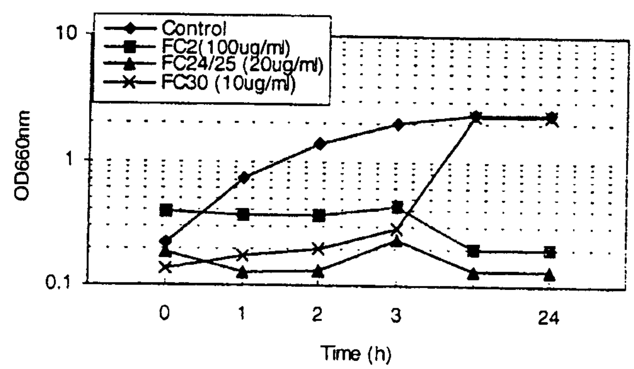

Figure 4

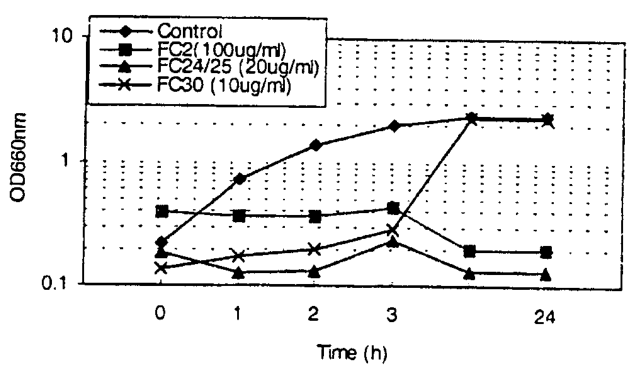

Figure 5

INTERNATIONAL SEARCH REPORT	International application No. PCT/AU 99/00284

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 31/34, A01N 43/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN CAS ON-LINE SUBSTRUCTURE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/29392 26 September 1996 See whole document, in particular page 2 lines 12-16, page 8 lines 31-33, Page 16 lines 2-3	1, 2, 4, 6, 7, 9, 12, 13, 15, 16, 17, 19
X	Chemical Abstracts 99: 200391 "Antimicrobial constituents (of marine algae)", Ochi Masamitsu, Suisangaku Shiriizu (1983), 45 (Kaiso no Seikagaku to Riyo), 101-19	1, 2, 4, 6, 7, 9, 12, 13, 15, 16, 17, 19

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 May 1999	13 MAY 1999

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929	S.R. IDRUS Telephone No.: (02) 6283 2536

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	De Nys, R.; Steinberg P.D.; Willemsen P.; Dworjanyn S.A.; Gabelish C.L.; and King R.J.; "Broad Spectrum effects of secondary metabolites from the Red Alga Delisea Pulchra in Antifouling Assays", Bifouling, 1995, Vol. 8, pp. 259-271. See in particular page 260 (third paragraph), pages 262-263 and page 265 (last paragraph).	1,2,4,6,7,9, 12,13,15, 16,17,19
X	Kotsuki Hiyoshizo; Monden Mitsugu; Ochi Masamitsu; "Efficient Synthesis of acetoxyfimbrolides and Beckerelides Analogs", Chemistry Letters, pp. 1007-1008 (1983) (The Chemical Society of Japan). See whole document, especially note "8".	1, 6, 12, 16

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/00284

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9629392	AU	49996/96	BR	9607661	CA	2215797
		CN	1185173	EP	815201	JP	11502108
		NZ	303630				

END OF ANNEX